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REVIEW

Sending Signals Dynamically

Robert G. Smock and Lila M. Gierasch

Proteins mediate transmission of signals along intercellular and intracellular pathways and between the exterior and the interior of a cell. The dynamic properties of signaling proteins are crucial to their functions. We discuss emerging paradigms for the role of protein dynamics in signaling. A central tenet is that proteins fluctuate among many states on evolutionarily selected energy landscapes. Upstream signals remodel this landscape, causing signaling proteins to transmit information to downstream partners. New methods provide insight into the dynamic properties of signaling proteins at the atomic scale. The next stages in the signaling hierarchy—how multiple signals are integrated and how cellular signaling pathways are organized in space and time—present exciting challenges for the future, requiring bold multidisciplinary approaches.

Transmission of signals between cells, within cells, and from the extracellular environment to the cellular interior is essential to life. In recent years, we have gained tremendous knowledge of the interacting networks that act as communication pathways for cellular signaling, culminating with extensive maps of "interactomes" based on genetic and physical interaction data [e.g., (*1*)]. Yet we know far less about how signals are passed from one component of a network to another. This puzzle can be viewed at the level of the protein machines that make up signaling networks: How does information, generally mediated by a binding interaction or a covalent modification, get relayed to the downstream member of the pathway?

It is increasingly apparent that signaling relies on the intrinsic dynamic properties of proteins and that proteins relay signals by shifting among different fluctuating energy states in response to one or more inputs. This emerging view of signaling raises many compelling questions: What is the genetic information that encodes the functionally productive dynamic properties of a protein? How do individual protein domains cooperate to form signaling pathways? How are signals integrated in multicomponent interconnecting networks? How do the dynamics of signaling proteins ultimately determine the response times of cells to signals and the time scales for signal propagation? We are beginning to develop the methods and principles to address these questions, but many challenges lie ahead. Our ability to develop therapeutic modulators of signaling and to reengineer cellular communication pathways will rely on progress in this fascinating but complex arena.

The intrinsic motions of proteins are determined by the covalent and noncovalent restraining forces that hold them together. The result is a symphony of dynamic modes oscillating at frequencies from picoseconds to milliseconds or even seconds. Tweaking a protein by a binding interaction or chemical modification alters this symphony, either gently changing its pitch or abruptly shifting the collective harmony. Just as the ability of a protein to fold is now understood to be best described by an "energy landscape," which maps the many states that a folding protein can visit as it samples conformational space en route to its native structure, so also is the ability of a signaling protein to respond to signals and pass them on dependent on the features of the energy landscape. The functional states crucial to signaling are in the lower energy regions of the overall folding landscape (Fig. 1). In both folding and signaling, the conformational states of a protein are populated to varying extents according to their energies, and rates of interconversions between states are governed by the heights of energy barriers between them.

Both the energetically favored structures of a given protein and its dynamic properties, including both amplitudes and frequencies of fluctuations between states, are encoded in its sequence and are subject to evolutionary pressures. Understanding how a given protein can transmit signals entails a full elaboration of its energy landscape and how this landscape is modulated by interactions with other proteins, peptides, or smaller ligands, as well as by covalent modifications such as phosphorylation. Thus, a static image of a protein, such as that from x-ray crystallography, is an extremely helpful starting point, but we must learn about the ensemble of accessible states in order to gain deeper insight into functions. For example, as simple a function as oxygen binding to hemoglobin requires a conformational change to enable oxygen entry and egress, which was noted by biophysicists as soon as they saw the structure (*2*).

It is considerably more difficult to fully describe the energy landscapes of proteins—both the extent of their sampling of different structures and the rates of this sampling—than it is to determine a single structure. Fortunately, research on protein dynamics and how functions of proteins relate to their movements has expanded greatly in recent years (*3*). Methodological advances that enable increasingly deep understanding of protein dynamics are emerging, concomitant with an enhanced awareness of complex interacting pathways in cellular physiology. Experimental methods that shed light on the dynamic properties of proteins are most informative in relatively narrow frequency ranges, and the methods applied to a given system must be matched to the underlying processes. Biological signal transmission occurs over a wide range of time scales. The fastest events are those triggered by light or electrical stimuli, which can take place on the femtosecond to picosecond time scales. Large-scale conformational rearrangements occur much more slowly, from milliseconds to seconds. Cellular networks of signals comprise molecular binding events, which may be transient or stable, and thus may prolong the time scale of signaling well beyond seconds.

The realization of the importance of dynamics to protein function is not new; major figures in biophysics recognized that protein motions were essential to function a half century ago (*4*–*7*). But progress in computational simulations of protein dynamics (*8*), in nuclear magnetic resonance (NMR) [in particular, relaxation dispersion (*9*)], and in single-molecule spectroscopy (*10*) has enabled the description of several signaling events in great depth on time scales from picoseconds to milliseconds. Together with an explosion of biological knowledge about complex signaling networks, these techniques offer an unprecedented opportunity for major advances in understanding. Below, we use several recently studied signaling systems to illustrate how the intrinsic dynamic properties of proteins allow them to act as switches and transducers in response to incoming signals and to thereby mediate signal transmission.

Incoming Signals Remodel Energy Landscapes

Signals can be transmitted by a shift in the equilibrium population of states for a protein with a rugged energy landscape (*7*). The "new view" of allostery (*11*) encapsulates the ideas of dynamics and postulates that the protein populates ensembles of many conformations at all times, fluctuating among these conformations. The interaction with a signaling partner remodels the landscape and consequently shifts the population distribution in such a way as to bias toward a particular downstream event.

How does a binding signal alter the energy landscape and lead to a productive signaling response? A central mechanism appears to rely on plasticity within an individual protein signaling domain—in other words, the existence of alternative residue packing networks with coupled dynamic motions. Extensive study of different examples of the widespread PDZ signaling

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domain has identified potential intramolecular structural and dynamic pathways that appear to connect incoming signals, notably binding to recognition motifs present on upstream partner signaling molecules, to downstream partners. In their many different cellular contexts, PDZ domains function to transduce these binding events into favorable domain-domain assembly of complexes.

The correlation of evolutionary conservation among PDZ domains pointed to a spatially contiguous set of residues as candidates for transmission of functional signals (*12*), and other methods including NMR dynamics analysis (*13*, *14*), thermal fluctuation analysis of crystal structures (*15*), and computational simulations of correlated motions (*14*, *16*, *17*) found similar networks (Fig. 2). Intriguingly, in one example (the second PDZ domain of human tyrosine phosphatase 1E), the network of residues showing similar binding-induced dynamic changes did not coincide with the set of residues that undergoes structural changes between ligand-bound and free states (*14*). An in-depth analysis of the dynamics time scales of another PDZ domain (from mouse tyrosine phosphatase BL) showed interconversion between different allosteric states to be relatively slow (microseconds to milliseconds). By contrast, a different PDZ domain (human PSD-95 PDZ3) lacked the dynamic network (*18*). These results, together with recent analysis using double-mutant cycles, support the notion that different PDZ domains evolve to have different dynamic properties tailored to their specific functions. In this case, PSD-95 PDZ3 functions as a rigid protein interaction domain (*19*).

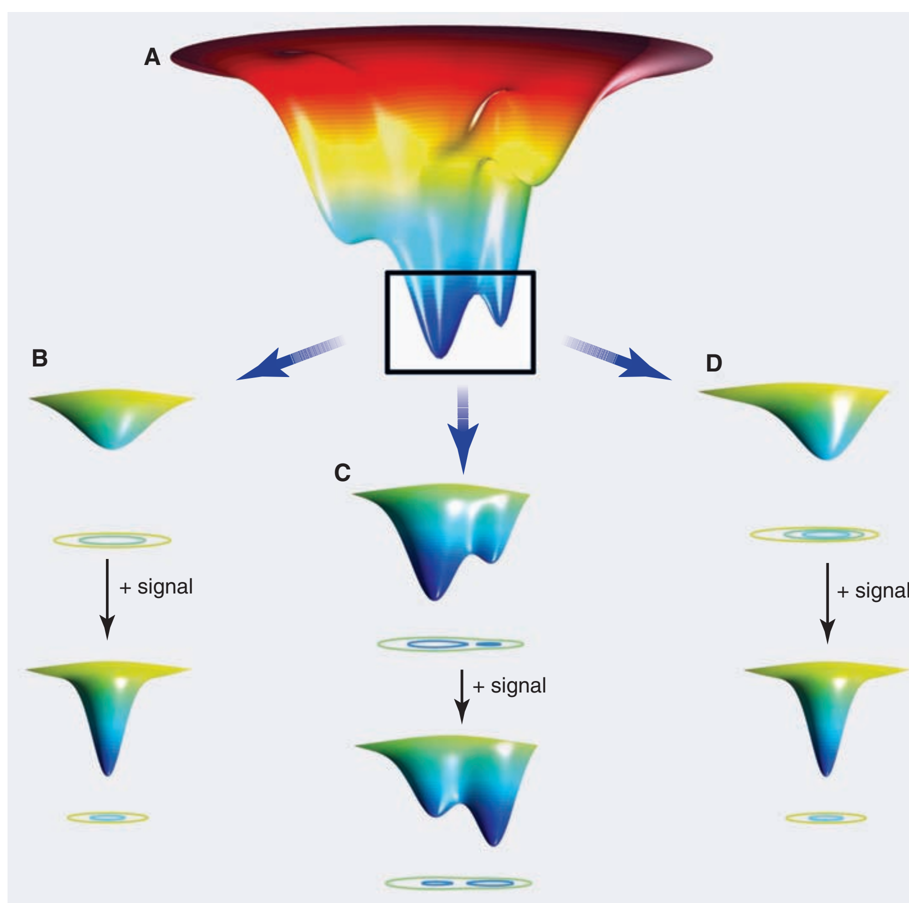

Fig. 1. Energy landscapes can be remodeled in several ways to alter protein dynamics and enable them to communicate signaling information. (**A**) Schematic illustration of the energy landscape available to a protein from higher energy (red) to lower energy (blue). Folding to the native state occurs as the large ensemble of non-native states moves down the energy funnel to the native state. The boxed region encloses conformational states that are energetically accessible and will be sampled under physiological conditions, given thermal fluctuations. (**B**) One way that a signal can remodel the energy landscape is to narrow the size of the ensemble of states in a single energy well. This reduces the dynamics of the protein, leading to a structural rigidification of the same average conformation. (**C**) Alternatively, a protein may exist in equilibrium between two distinct conformational states, and an incoming signal can alter the relative energies of the two states, leading to a redistribution of their occupancies. (**D**) A slight variation on (C) may occur if the sampling of a higher-energy state in the absence of ligand provides a partial pathway toward a signal-induced conformation, as shown by partially overlapping wells of the two states. In the landscape shown, the higher-energy state is narrowed and shifted somewhat in structure upon interaction with a signal.

Whereas intramolecular signal transmission in PDZ domains seems to arise from a combination of structural changes and dynamic fluctuations, other domains rely more exclusively on dynamics for signaling (*20*). For example, the phosphotyrosine-binding domain of insulin receptor substrate–1 (IRS-1) binds to the autophosphorylated state of hormone-activated insulin receptor to mediate downstream signaling. A detailed NMR dynamics analysis revealed only subtle conformational changes between free IRS-1 and IRS-1 bound to a phosphotyrosine-containing peptide (*21*). Rather, a cluster of dynamically perturbed residues was found to connect the peptide-binding site to a distal surface, where the subsequent downstream signaling interaction was postulated to occur. In another example (*22*), binding of one cyclic adenosine monophosphate (cAMP) to CAP, a homodimeric cAMP-binding transcriptional activator, reduced the affinity for the second cAMP without any alteration in the overall structure of the subunit but with enhancement of its overall mobility on a microsecond to millisecond time scale. By contrast, binding of the second ligand led to rigidification (Fig. 1B). The authors provide a compelling case that the altered dynamics and consequent entropy costs explain the observed negative cooperativity in cAMP binding with no change in the average structure of the CAP protein.

Biological signaling requires the integration of multiple inputs: Multiple interactions can be modulated within the same molecule by coupling the remodeling influences of more than one ligand. For example, calmodulin, a central player in intracellular calcium signaling, regulates a wide array of downstream partner proteins in response to calcium concentration. A recent NMR study showed that binding to different target regulatory domains alters calmodulin internal dynamics to differing extents and "tunes" affinity through conformational entropy (*23*). Moreover, calmodulin signaling is dependent on its rugged energy landscape with multiple low-energy valleys and consequent ability to undergo substantial conformational rearrangement. The relatively small calmodulin molecule links two calcium-binding EF hands by a long helix that has the capacity to fold back on itself in a hinge-like motion. A recent study (*24*) computationally elaborated the ensembles of structures sampled by calmodulin in its free and calcium-bound states and showed, by incorporating NMR-derived distances and orientation parameters, how this ligand alters dynamics locally (i.e., within the EF hand subdomains) (Fig. 3A). These local events increase the affinity for downstream signaling molecules,

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such as myosin light chain kinase, which first binds the C-terminal calcium-bound EF hand. This binding in turn predisposes the N-terminal domain to form additional contacts, thus causing the overall molecular hinge to close. The result is a clamping of the partner molecule between the two halves of calmodulin. The findings are consistent with previous NMR (*25*) and single-molecule fluorescence results (*26*); together with other recent studies of this system (*27*, *28*), they illustrate how the intrinsic dynamics of a molecule such as calmodulin can lead to multistep coupled allosteric signaling events.

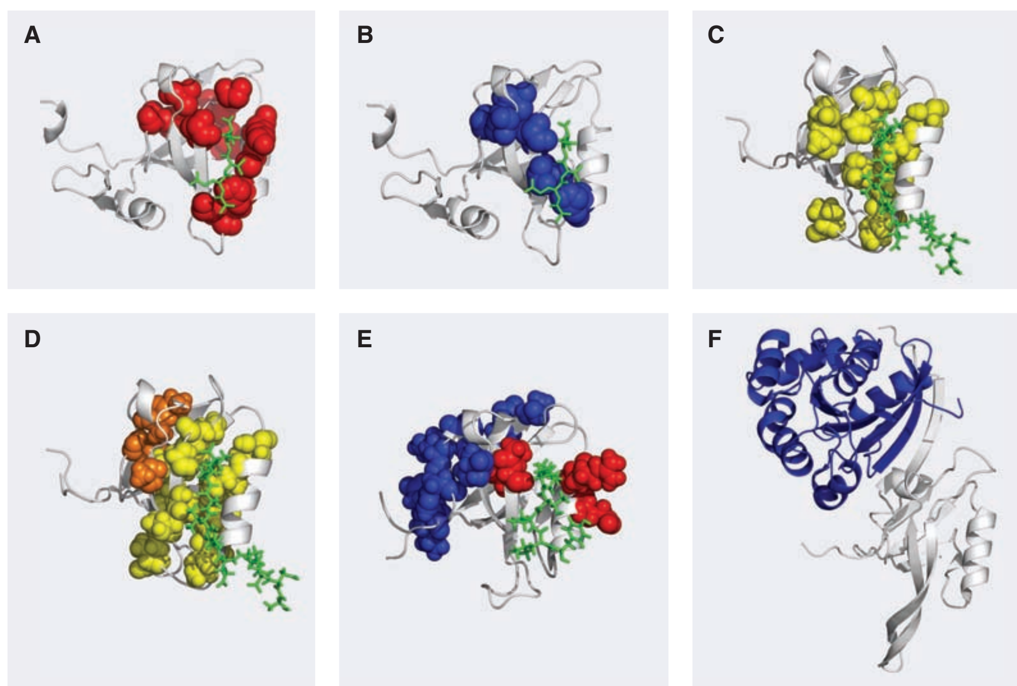

Fig. 2. Potential pathways of signal transmission within isolated PDZ domains. Similar networks linking the peptide-binding site (bound peptide shown in green sticks) to distal surfaces have been identified in PDZ domains by a number of approaches. (**A**) Patterns of evolutionarily coupled residues conserved among PDZ family members (shown in red spacefill) connect the peptide-binding site to a distal surface of the domain (*12*). (**B**) A network of residues identified by analysis of thermal fluctuations in PDZ-domain crystal structures is shown in blue spacefill, with the bound peptide in green sticks (*15*). (**C**) A pathway comprising residues in a PDZ domain, showing dipeptide-induced changes in their side-chain dynamics, is shown in yellow spacefill, with the peptide ligand in green sticks. (*13*) (**D**) A dynamic network identified by a molecular dynamics simulation restrained by experimental parameters (*14*) is shown in spacefill. Unexpectedly, one portion of the network shows enhanced flexibility upon peptide binding (orange) and, conversely, another region (red near the peptide binding pocket) shows reduced dynamics. (**E**) Domain-domain interaction between two PDZ domains appears to make use of the same basic pathways identified within an isolated PDZ domain. Residues shown as blue spheres were directly implicated in interdomain interactions in a phosphotyrosine phosphatase BL PDZ domain, and a set distal to these showed increased dynamics (red spheres) (*42*). (**F**) The interdomain interface identified in a crystal structure (PDB code 1NF3) of a complex of the regulatory Cdc42 (blue cartoon) and the Par-6 PDZ domain (white cartoon) also coincides with the surface of the PDZ domain that is connected to the peptide binding site by many of the identified intradomain networks.

Autoinhibition via a Reversible Intramolecular "Latch"

Several signaling proteins are autoinhibited via internal domain-domain interactions. An upstream signal, such as a binding or phosphorylation event, leads to destabilization of the internal contacts, releasing the autoinhibition and enabling downstream events. In a landscape view, the inhibited signaling protein populates predominantly the autoinhibited state but visits occasionally excited states where inhibition is relieved. The ability of ligands to modulate this equilibrium and the dynamic nature of the response to a signal have now been quantitatively established for the human proto-oncogene Vav, which relays signals from cell surface receptors to modulate intracellular events, by combined use of NMR relaxation methods and biochemical assays (*29*). In this case, autoinhibition occurs because an acidic region (the "latch," termed Ac) binds as a helix to the guanine nucleotide exchange factor (GEF) substrate-binding site of the Vav Dbl homology (DH) domain (Fig. 3B). An excited state, in which the acidic helix is displaced, was sampled on the microsecond to millisecond time scale in the absence of any upstream signal. Phosphorylation of a single tyrosine in Ac (Tyr^{174}) catalyzed by an upstream kinase led to release of Ac binding and a shift of the population to the state that was previously infrequently visited (Fig. 1C). Strikingly, Tyr^{174} is not accessible in the inhibited state of Vav DH. Thus, the population of the excited state limits the rate of phosphorylation; moreover, for the mutant proteins that were amenable to this measurement, the excited-state population also paralleled their ability to activate the downstream GEF. When phosphorylated, the Ac region lacks stable secondary structure and is quite flexible. The Ac of Vav epitomizes "intrinsically disordered regions" (IDRs), which have evolved to be devoid of stable structure and often play key roles in signaling (see below) (*30*).

Autoinhibition-based switches can be regulated extrinsically, as by phosphorylation of the Vav Ac domain, or intrinsically by the timing of a conformational change required to release the "latch." The Crk protein, an intracellular regulator of the tyrosine kinase Abl, comprises an Src homology 2 (SH2) domain followed by two SH3 domains separated by a 50–amino acid linker. The N-terminal SH3 domain of Crk binds proline-rich motifs in target proteins but is autoinhibited by interaction with the C-terminal SH3 domain. This inhibition is dependent on the Gly^{237}-Pro^{238} peptide bond within the linker adopting the cis isomeric state (*31*). A slow isomerization to the trans form (on a time scale of seconds) releases the interdomain linker and opens the N-terminal SH3 domain polyproline II ligand-binding site to partner interactions. Although these downstream aspects remain unknown, the cis-trans equilibrium may be regulated by Abl-mediated phosphorylation of a tyrosine near the key Gly^{237}-Pro^{238} bond in the linker, and the rate of interconversion between cis and trans forms can be catalyzed by prolyl isomerases. In turn, regulated access to this latter enzyme could afford yet another layer of dynamic regulation.

Disordered Regions Are Built-In Dynamic Switches

A large fraction of the exons in the human genome carry the signature amino acid composition and patterns predicted to fall into the class of IDRs [or IDPs (intrinsically disordered proteins)], and these regions are frequently reported to function as dynamic entities in signaling (*30*, *32*–*34*). By negative design, these regions retain the ability to interact with multiple recognition sites, typically

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in alternative conformations. Some IDRs have the intrinsic ability to serve as molecular recognition features. In some cases, such as the Vav Ac described above, these regions may interact with another domain or protein in a stable secondary structure some of the time, and may then switch to another state that is either disordered or bound to a second binding partner. Their binding affinities are governed by competing entropic contributions (the costs of ordering the region upon binding) and enthalpic contributions (the favorable interactions formed). In addition to their malleable nature, IDRs provide geometric flexibility, allowing domain movements and serving as variable-length dynamic tethers.

The ability of IDRs to bind multiple partners leads to functional diversity in signaling cascades, as illustrated by the IDR-containing cell cycle regulator p21 (*35*) (Fig. 3C). Additionally, in this same group of cell cycle regulators, p27 illustrates that IDRs can bridge across long distances in complexes, in this case between a site on cyclin-dependent kinase (Cdk)–and one on cyclin A (*36*). Evidence from NMR suggests that binding to IDRs may occur stepwise, facilitating a "fly-casting" search, whereby multiple weak binding events favor initial complex formation (*37*, *38*) (Fig. 3D). Subsequent "Velcro"-like multivalent binding using several interactions at once leads to stable complexes. This coupling of binding and folding is accompanied by an entropically uphill "disorder-to-order" transition. In an alternative manifestation of multivalent binding, the recognition of the Cdk inhibitor Sic1 by its receptor, Cdc4, occurs via binding of several different sites on Sic1 to one site on the receptor, in a fluctuating dynamic equilibrium (*39*) (Fig. 3E). In this example, linking several weakly binding sites on one dynamic ligand maintains its receptor in an activated state. Clearly, the requirements for any given signaling interaction can be "tuned" by means of dynamic and intrinsically unstructured binding motifs.

Switchable Domain-Domain Interactions

Signaling network complexity can be achieved by flexibly linking domains and coupling signal-induced intradomain structural and dynamic responses to overall domain-domain rearrangements. This strategy accounts for the "Lego"-like evolutionary diversification of signaling pathways by recurrent use of the same switchable modules (*40*, *41*). This hierarchical buildup of signaling complexity is exemplified by the PDZ domains described above, which exist in combinations in many signaling proteins (Fig. 2). Their responses to signals can be altered by their context. For example, the first PDZ domain of mouse phosphotyrosine phosphatase BL (which contains five PDZ domains) modulates the

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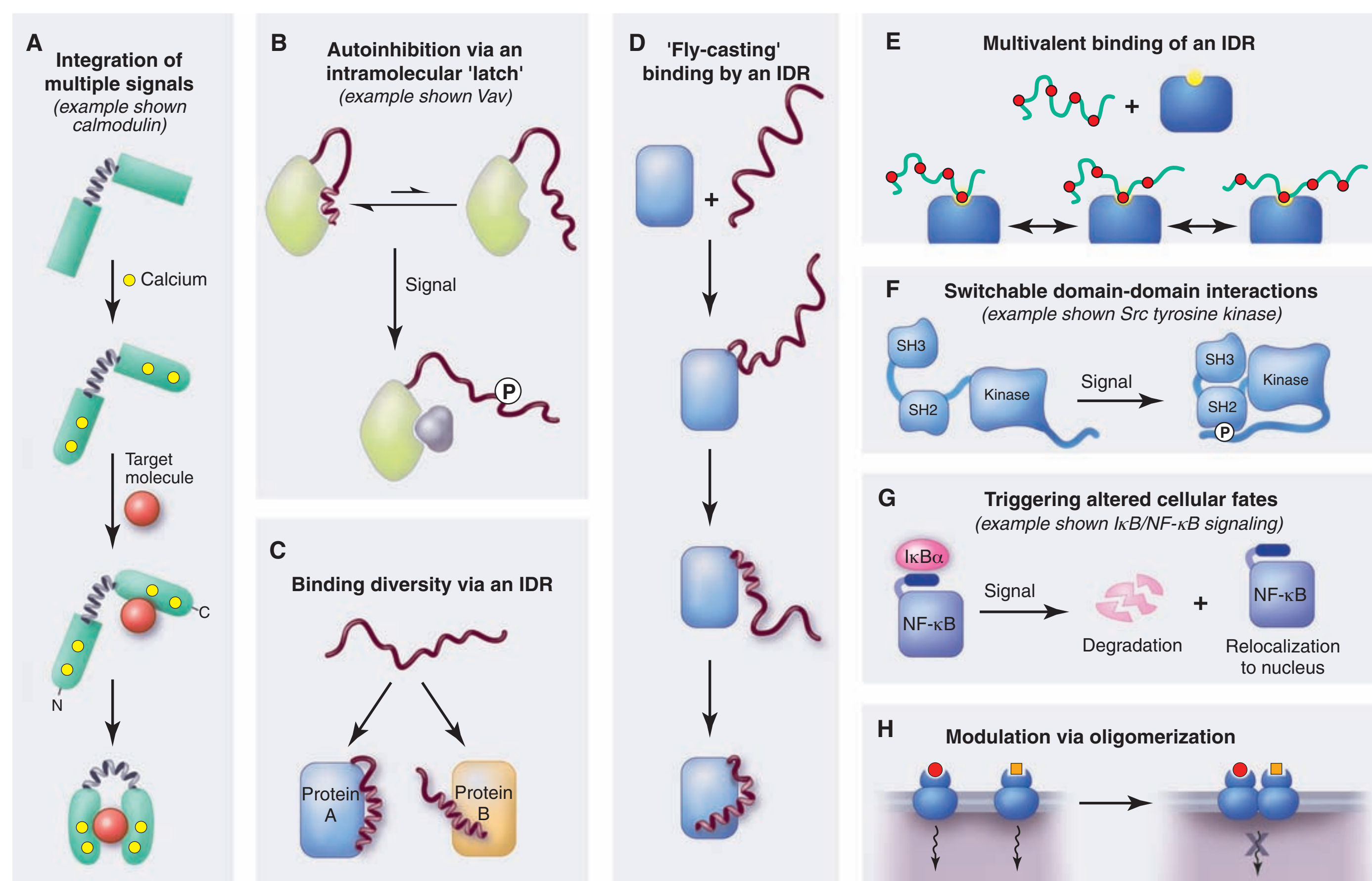

Fig. 3. Examples of how proteins take advantage of intrinsic dynamic properties to respond to an incoming signal. (**A**) Multiple signals can be integrated to create a response. Here, in the case of calmodulin, first calcium (yellow circles) is sensed by binding to the EF hand subdomains, which favors binding to a target molecule such as myosin light chain kinase (red circle). Binding of the target to one site on calmodulin leads to enhanced likelihood of binding to a second binding site (*24*). (**B**) Intramolecular autoinhibition can occur such that a downstream target interaction site is occluded. Opening of the autoinhibitory domain (here shown for Vav) can be favored by an upstream signal such as phosphorylation (*29*). (**C**) An intrinsically disordered region (IDR) can confer binding diversity on a signaling protein, enabling two targets to be recognized (*35*). (**D**) IDRs also enable a "fly-casting" mechanism of binding by allowing stepwise association with a target (*37*). (**E**) In some cases, multivalent binding can be mediated by an IDR that harbors several potential binding sites (small red circles) that each transiently occupy a single site on the target (*39*). (**F**) Dynamic domain-domain rearrangements can be triggered by signals such as phosphorylation (*45*). (**G**) Disruption of complexes can reveal signals for any of several downstream outcomes, such as relocalization in the cell and degradation (*47*). (**H**) Receptor complexes enable the possibility of higher-order modulation of responses to two different signaling ligands (shown as red circle and yellow square) (*50*).

peptide affinity and specificity of its second PDZ domain through an interdomain interaction site that is consistent with the intradomain networks discussed above (*42*). Similarly, the *Drosophila* cell polarity protein Par-6 is regulated by the Rho guanosine triphosphatase (GTPase) Cdc42 via binding of Cdc42 to a CRIB domain adjacent to the Par-6 PDZ domain. Cdc42-CRIB activation of the PDZ domain occurs with pronounced rigidification of CRIB and CRIB-PDZ contacts on the opposite side from the PDZ ligand-binding site, consistent with the signaling pathways observed for isolated PDZ domains. This indirect domain-domain rearrangement changes the conformation of the PDZ domain, increasing its affinity for peptide and triggering subsequent cell polarity signaling (*43*). PDZ conformational switching has also recently been implicated in the INAD scaffold protein of the *Drosophila* visual photoreceptor (*44*); this protein was previously thought to be a passive organization template. The new data show that INAD PDZ5 acts as a redox switch, switching an internal cysteine pair to an oxidized state in a light-dependent manner to regulate the binding of target proteins and promote signaling, although the mechanistic details of signal transduction are unclear.

Incoming signals can also promote assembly and disassembly of multiple domains (Fig. 3F). Src tyrosine kinase SH2 and SH3 domains undergo reversible assembly and disassembly in a dynamic equilibrium that is dependent in part on binding of a phosphotyrosine from the adjacent kinase domain. Binding of the phosphorylated kinase segment to the SH2 domain favors SH2-SH3 assembly and inactivates the kinase. When the kinase is activated (and the tyrosine is not phosphorylated), the SH2-SH3 tandem domains are uncoupled from one another. They nonetheless fluctuate between the assembled and disassembled arrangements. The interdomain linker is crucial to this fluctuating equilibrium, as mutation of the linker can lead to constitutive activation (*45*). Apparently, the interdomain linker biases substates populated in the active form of Src kinase so as to reduce conformational search in assembly-mediated inactivation (Fig. 1D). Switchable domain-domain associations involving the interdomain linker are also exemplified by transmembrane C-cadherin proteins, which function in cell adhesion during development (*46*). Binding of calcium rigidifies flexible segments between cadherin repeats, altering their dynamics and mechanical properties and increasing the availability of adhesive contacts.

Modulation of dynamics and domain arrangements from incoming signals can lead to altered cellular localization and enhanced degradation (Fig. 3G). The NF-κB transcription factor is normally bound to the inhibitory protein IκBα, which partially occludes a NF-κB nuclear localization sequence, resulting in dynamic shuttling between the cytoplasm (predominantly) and the nucleus. Relief of inhibition occurs through phosphorylation, ubiquitination, and consequent degradation of IκBα, which results in localization of NF-κB exclusively to the nucleus and transcriptional activation of target genes, including IκBα, which acts via negative feedback. Several of IκBα's ankyrin repeat domains rigidify upon binding to NF-κB, but there is an increase in flexibility in the central repeats (*47*). The IκB–NF-κB signaling module has emerged as a paradigm for linkage of regulation of gene expression to temporal responses of a signal transduction network, and its behavior has been mathematically modeled by several groups (*48*). An exciting future prospect is the correlation of the molecular dynamic properties of its components to the signaling dynamics of the entire system.

Wholesale Intermolecular Reorganization: Switchable Oligomerization and Arrays

Cellular networks display yet higher levels of organization. Recent studies show that oligomerization and array formation of signaling molecules, and of transmembrane receptors in particular, can have important functional consequences in several systems. Arrays of transmembrane receptors can integrate a spectrum of coincident extracellular signals, and the dynamics of formation and disassociation of the arrays will alter the cell's response to the multiple inputs. Examples are provided by bacterial chemotactic receptors, which cluster in heterogeneous arrays, "sniffing" the extracellular environment for any of several chemotactic signals and altering cell motility. The intracellular response elements are in large measure shared among the chemotactic receptors, even though they respond to different extracellular ligands. A recent elegant study shows clearly that clustering density has a marked effect on intracellular kinase and methylation activities (*49*).

The superfamily of heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptors (GPCRs), which comprises many subclasses that respond to specific ligands and perform a myriad of signaling functions, can show even greater functional diversity through multimerization. Cross-talk between the monomers within homodimers of GPCRs has been described, and newly discovered functional consequences of assemblies of different GPCRs are emerging. In one case, the binding of morphine to the μ-opioid receptor induces conformational change in a bound α_{2A}-adrenergic receptor, which in turn inhibits activation of its associated G protein and downstream signaling under conditions that would promote signaling in the absence of either morphine or receptor dimerization (*50*) (Fig. 3H). A fascinating extension of this theme involves association of various receptor types into a higher-order functional mosaic: Functional clustering of cannabinoid, dopamine, and adenosine receptors was indicated by the observation that ligands for the adenosine receptors modulated interactions between the cannabinoid and dopamine receptors (*51*). Such observations underscore the complexity of functional relationships in signal integration and propagation.

Another example of modulation via oligomerization was recently described for the Wiskott-Aldrich syndrome protein (WASP) family (*52*), members of which control actin dynamics through stimulation of Arp 2/3 complexes. The WASP VCA domain is responsible for the Arp 2/3 interaction but is normally intramolecularly inhibited by association with an adjacent GTPase-binding domain (GBD). This autoinhibition is allosterically relieved by a variety of WASP activators, which disrupt the interdomain contacts and free the VCA domain for its downstream interactions. WASP dimerization greatly enhances the affinity of the VCA domain for Arp2/3, leading to localized signal amplification. Dimerization is postulated to be favored by higher-order effects (such as interaction with multivalent WASP-binding partners) or by membrane clustering mediated by binding to regions enriched in phosphatidylinositol 4,5-bisphosphate. Interestingly, WASP and the related family member WAVE can heterodimerize in some cooperative processes, enabling yet more complex signal integration via allosteric and oligomeric mechanisms and increasing the capacity of the system to receive and integrate a greater variety of signals.

Perspective: From Atomic-Resolution Domain Dynamics to Complex Signaling Networks

Cells and organisms are processing and reacting to many signals at all times. The integration of many signals by complex networks is central to balancing needs and coordinately regulating a multitude of biochemical pathways. We have attempted to illustrate how the fundamental dynamic properties of signaling proteins may enable them to mediate these complex signaling tasks. New advances are offering greater insight into intrinsic dynamic properties of small protein modules and how binding events and covalent modifications influence them. Concurrently, explosive progress in the identification of components in signaling networks and the mapping of their interactions provides a tantalizing challenge for the future: Can we connect the atomic-scale descriptions of protein dynamics to the higher-level interdomain and intermolecular communication events that make up a signaling network?

Paradigms for how proteins act as receivers, switches, relays, and nodes in pathways are emerging. A hint of how higher-level organization of signaling components can lead to integration of signals and coordinated modulation of responses has been provided in a few cases. However, there remains a huge gap between the molecular level and the cellular or intercellular

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level of signaling. This gap is spatial (cellular distances are on the scale of micrometers; protein movements are measured in angstroms), organizational (compartmentalization and orderly arrangements of pathways are essential to life), temporal (molecular movements occur in picoseconds to seconds, whereas cellular communication may persist substantially longer), and combinatorial (the sheer numbers of cell types, proteins, small molecules, stimuli, etc., and the multiplicity of their functional relationships with each other lead to extraordinary numbers of possibilities). The challenge to bridge this gap will require powerful new methods, interdisciplinary strategies, and creative, bold minds. We are encouraged by efforts to apply information theory to quantitatively interpret signal transmission (*53*), by multiscale modeling to bridge the molecular simulations to biochemical networks (*54*), and by whole-cell mapping of signaling protein dynamics (*55*, *56*). A holistic picture of the entire orchestra of dynamic contributions to cellular signaling can now begin to be envisioned.

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REVIEW

Protein Dynamism and Evolvability

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The traditional view that proteins possess absolute functional specificity and a single, fixed structure conflicts with their marked ability to adapt and evolve new functions and structures. We consider an alternative, "avant-garde view" in which proteins are conformationally dynamic and exhibit functional promiscuity. We surmise that these properties are the foundation stones of protein evolvability; they facilitate the divergence of new functions within existing folds and the evolution of entirely new folds. Packing modes of proteins also affect their evolvability, and poorly packed, disordered, and conformationally diverse proteins may exhibit high evolvability. This dynamic view of protein structure, function, and evolvability is extrapolated to describe hypothetical scenarios for the evolution of the early proteins and future research directions in the area of protein dynamism and evolution.

Proteins are proficient, accurate, and specific. These characteristics generally correlate with a lack of versatility; however, proteins also exhibit a marked ability to acquire new functions and structures. The evidence for the evolutionary adaptability of proteins is compelling, not only in the vast range of proteins that have presumably diverged from a few common ancestors, but also in recent evolutionary events such as the emergence of drug resistance and enzymes that degrade chemicals that appeared on this planet only a few decades ago.

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What are the features that make proteins evolvable? Evolution acts by enriching pre-existing diversities. Proteins conforming to the traditional view of absolute functional specificity, and only one well-defined structure are therefore not likely to readily respond to new selection pressures. However, a "new view" of proteins as an ensemble of alternative substructures, or conformers, in equilibrium with their so-called "native state" currently prevails [the new view was originally proposed by R. L. Baldwin and K. A. Dill in relation to protein folding and was later extended to describe native state ensembles (*1*)]. The new view is more consistent with evolutionary adaptability and is extended here to an avant-garde view of protein dynamism and evolvability.

Conformational variability, or dynamism, is an inherent property of any polymeric chain. The conformational diversity observed in proteins ranges from fluctuations of side chains and movements of active-site loops to secondary structure exchanges and rearrangements of the entire protein fold. Alternate structural conformers can mediate alternate folds and functions (*1*, *2*). Such structural and functional diversity is the foundation of "protein evolvability," defined as the abil-